β-N-methylamino- L-alanine (BMAA) produced by cyanobacteria as a possible cause of neurodegenerative diseases

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INTRODUCTION

Neurodegenerative diseases are disorders of the central nervous system that result from the selective and premature atrophy of functionally related neurons.
Although each of these diseases has early-onset familial forms, the vast majority of cases are sporadic, supporting the notion that environmental factors can be an important cause of neurological disorders although this fact is usually undervalued.

Cyanobacteria are a phylum of photosynthetic bacteria characteristic for cyanobacterial blooms formation, their ubiquity and their ability to produce a wide range of potent hepatotoxins, neurotoxins, cytotoxins and inflammatory agents which can affect public health.

Although most articles talk about BMAA as a neurotoxin produced by members of all five cyanobacterial sections including symbionts and free-living as well, some articles refuse this idea and affirm that since this non-protein amino acid has antiherbivory properties, it should take part as a component produced by plants.

Moreover, some authors have linked BMAA with amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and Parkinson's disease (PD) and diverse experiments in vitro as well as in vivo have been made in order to demonstrate it, while others deny this hypothesis owing to non detection BMAA having been reported in their studies.

The aim of this work is to review: if cyanobacteria produce the neurotoxin BMAA and if this non-protein amino acid is actually a possible cause of neurodegenerative diseases

RESULTS

a) Experiments in order to know if cyanobacteria produce the neurotoxin BMAA

1

Symbionts cyanobacteria

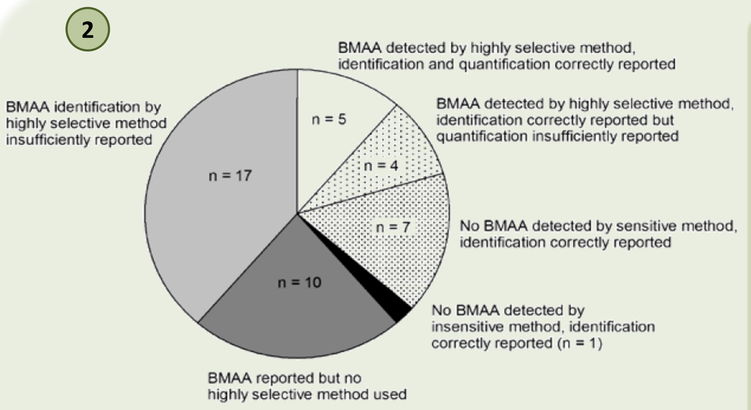
Free living cyanobacteria

Cyanobacteria	Host	Symbiont	Free BMAA, μg/g	Protein BMAA, μg/g
Nostoc PCC 9305	Hornwort	*Anthoceros*	156	1,400
Nostoc PCC 7422	Cycad	*Cycas*	ND	962
Nostoc 8001	Flowering plant	*Gunnera monoica*	203	664
Cyanobacterial species/strain	**Section***	**Habitat**	**Free BMAA, μg/g**	**Protein BMAA, μg/g**
Myxosarcina burmensis GB-9-4	II	Marine coral	79	1,943
Planktothrix agardhii NIES 595	III	Freshwater	318	30
Fischerella PCC 7521	V	Yellowstone, hot spring	44	175

Table 1. BMAA is produced by diverse taxa of cyanobacteria in free living as well as symbionts. Ref. [4]

Free living cyanobacteria produce less doses of BMAA than when stay in symbiosis with collaroid roods. Moreover, concentration of BMMA in protein form is higher than free form.

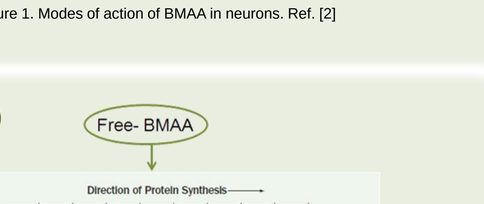
2



Graphic 1. Differences on identification of BMAA showing that not always results are positive. Ref. [6]

Despite in most articles BMAA is found in cyanobacterial cultures, there are some studies that have not provided conclusive evidence of it because different methods were used, or they may contain errors due to a lack of critical discussions and revision.

3



BMAA is produced within cyanobacteria in response to nitrogen starvation

Graphic 2.Observation of BMAA depending on growth conditions. Ref. [5]

In this experiment has been demonstrated that BMAA is produced in axenic cyanobacterial culture with deprivation of nitrogen. It is suggest that BMAA is produced by cyanobacteria and that it may have a function in nitrogen metabolism .

b) Experiments so as to determine if BMAA is one possible cause of neurodegenerative diseases:

1

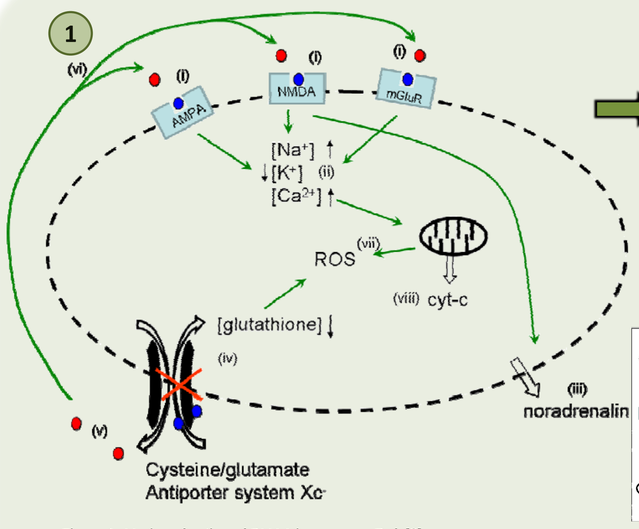
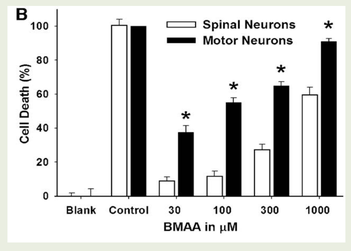
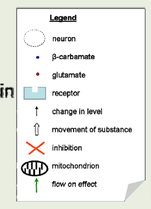


Figure 1. Modes of action of BMAA in neurons. Ref. [2]



Graphic 3. Impact induced by different BMAA's concentrations on spinal neurons and motor neurons. Ref. [8]

Motor neurons (MN) are more susceptibility to low concentrations of BMAA than other neurons. BMAA triggers considerable damage in MN while leaving spinal neurons relatively uninjured. This fact is related to the pattern of selective neuronal loss seen in ALS



When BMAA is present as a β-carbamate it can bind to NMDA, AMPA and mGlu receptors. This activation results in an increase of Na+ and Ca2+ and a decrease of K+ channeling the release of ROS. Cell becomes depolarized and leads to the permeability of the cell membrane and the release of noradrenalin. BMAA also inhibits the cysteine/glutamate antiporter system Xc^- resulting in glutathione depletion which contributes to rises in oxidative stress. System Xc^- also increases extracellular glutamate which can then bind glutamate receptors and extend the damage by excitotoxicity.
Finally, cytochrome-c is released from the mitochondria resulting in the induction of apoptosis.

2

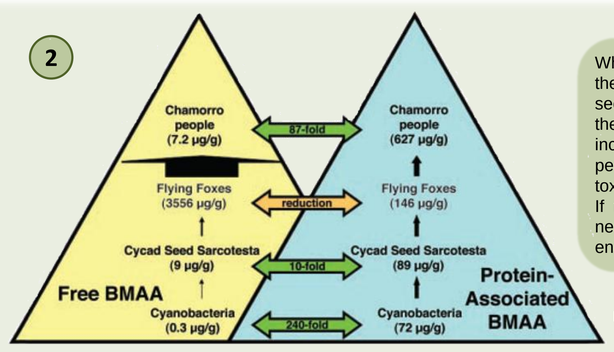
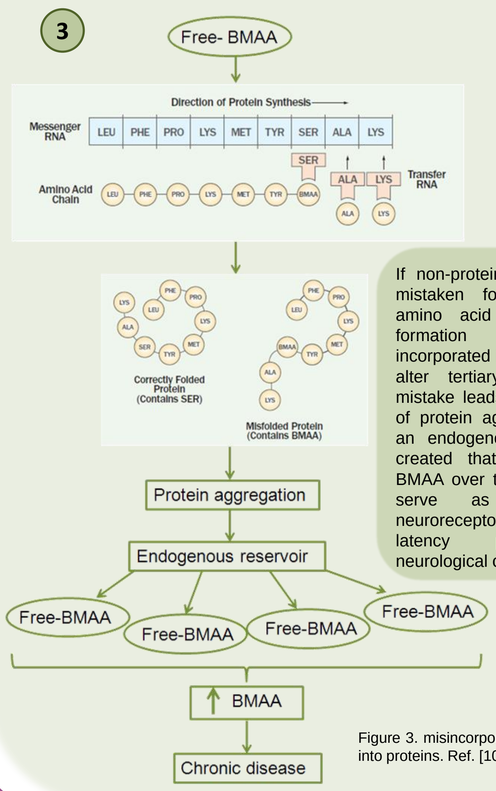


Figure 3. Biomagnification of BMAA into Guam ecosystem. Ref. [1]

When cyanobacteria stay in symbiosis with Cycas, the neurotoxin is localized on the sarcotesta of seeds. Flying foxes as well as other animals fed on these seeds bioaccumulate BMAA into their tissues increasing that concentration. When Chamorro people eat this flying foxes they biomagnificate the toxin into their brain and develop the disease.
If this chain didn't exist, BMAA wouldn't cause neurological dysfunctions because there aren't enough doses in the environment to cause disease.



If non-protein amino acid is mistaken for an essential amino acid during protein formation it can be incorporated into proteins and alter tertiary folding. This mistake leads to an increase of protein aggregation. Then an endogenous reservoir is created that releases free-BMAA over time. This BMAA serve as agonist at neuroreceptor and cause latency periods and neurological chronic diseases.

Figure 3. misincorporation of BMAA into proteins. Ref. [10]

4

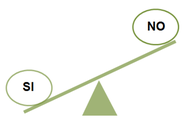
Route of exposure	Species	Dose level, exposure time	Research group and date	Observations
Intraperitoneal injections	Rat Chicken	6–14 μmoles/g body weight 3–7 μmoles/g body weight	Vega and Bell. 1967	Weakness, convulsions and uncoordination
Gavage	Monkey	100–350 mg/kg daily, up to 10 weeks	Spencer *et al.* 1987	Corticomotoneuronal dysfunction, Parkinsonian features and behavioural abnormalities
Dosed feed pellets	Mouse	28 mg/kg daily, 30 days	Cruz-Aguado *et al.* 2006	No motor, cognitive or neuropathological effect observed

Table 2. A chronological summary of mechanisms of BMAA activity in vivo. Ref. [2]

Experimental model	Species	Dose level, exposure time	Research group and date	Conclusion
Minced brain	Rat	5 mM, acute	Brownson *et al.* 2002	Impairment of intracellular calcium ion homeostasis. Possible neuronal death.
Primary embryonic spinal cord culture	Mouse	30–1000 μM, 20–24 h	Rao *et al.* 2006	Effects on calcium dependent cascades Increase on calcium ion concentration and ROS. Selective damage to motor neurons
Primary mixed cortical cell cultures	Mouse	3 mM, 3 h	Liu *et al.* 2009	Induction of oxidative stress is through inhibition of the cystine/glutamate antiporter system Xc^-

Table 3. A chronological summary of mechanisms of BMAA activity in vitro. Ref. [2]

Results show a strong relation between biochemical and neuronal functions changes depending on the diverse doses of BMAA and exposure times.



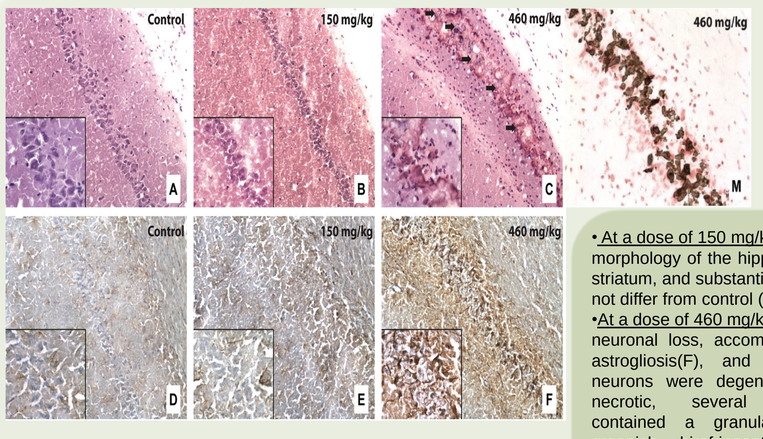


Figure 4. Direct histological observations of hippocampal sections of adult rats neonatally treated with different doses of BMAA. Ref. [7]

• At a dose of 150 mg/kg: morphology of the hippocampus, striatum, and substantia nigra did not differ from control (B and E).
•At a dose of 460 mg/kg: neuronal loss, accompanied by astrogliosis(F), and remaining neurons were degenerating or necrotic, several neurons contained a granular, faintly greenish, birefringent material (C) and presence of calcium deposits (M) was demonstrate.

6

Non-neurological disease controls		
12 cases		
	22 specimens	none detected
	2 specimens	in two different brains – BMAA 36 and 45 μg/g
Huntington's disease controls		
8 cases		
	7 specimens	none detected
	1 specimen	BMAA 11 μg/g
Alzheimer's disease		
12 cases		
	23 specimens	BMAA detected, mean 111±15 μg/g
	1 specimen	none detected
ALS		
13 brains		
	26 specimens	BMAA detected, mean 134±13 μg/g
4 spinal cords		
	4 specimens	BMAA detected, mean 124±69 μg/g
Parkinson's disease		
2 brains		
	2 specimens	BMAA detected in both, 176 and 218 μg/g

Levels of BMAA present in brain tissue of people who died due to neurodegenerative diseases is a relevant evidence that this neurotoxin is strictly related with this type of pathologies.
However, BMAA was not detected in HD patients and the bond between BMAA and all types of neurodegeneratives can be rejected.

Table 3. Analysis post-mortem of brain from people who died as a consequence of neurological diseases. Ref . [1]

DISCUSSION AND CONCLUSIONS

- Most studies seem to indicate that cyanobacteria produce BMAA in spite of negative results. Possible explanations for the striking variations in BMAA concentrations could be the variance in the range of BMAA content between genera and species and also within the ratio of free to protein-bound BMAA suggests that BMAA production and storage depends on growth conditions and life cycle stages.
- All results seem to indicate that BMAA cause neurodegeneration but divergence in opinions is still evident. A possible explanation for the variations on presence / absence of BMAA in the same tissue could be attributed to the use of different animal models and the level of impact and susceptibility of each specie. Differences in the pharmacokinetics and distribution of BMAA or the need of longer time of exposure may be crucial to observe neurological deficits. Other factors that should influence are the analysis with different methods that yield diverse results, specially when using a non-selective method or others that are not as sensitive, and last but not least, not revising the works.
- An animal model of chronic BMAA toxicity is needed, not only to prove the concept of the hypothesis but also to provide a test-bed for developing therapeutic strategies.
- Discovering that BMAA is produced by diverse taxa of cyanobacteria and their ubiquity, suggests that exposure to BMAA may be more widespread than previously believed and that it is necessary a severe control in order to prevent diseases.

REFERENCES

[1] **Bradley, W. G., & Mash, D. C (2009).** *Amyotrophic lateral sclerosis : World Federation of Neurology Research Group on Motor Neuron Diseases*, *10 Suppl 2*(August), 7–20.
[2] **Chiu, A. S., Gehringer, M. M., Welch, J. H., et.al (2011).** *International journal of environmental research and public health*, *8*(9), 3728–46.
[3] **Cox, P. A., Banack, S. A., & Murch, S. J (2003).** *Proceedings of the National Academy of Sciences of the United States of America*, *100*(23), 13380–3.
[4] **Cox, P. A., Banack, S. A., Murch, S. J., et.al (2005).** *Proceedings of the National Academy of Sciences of the United States of America*, *102*(14), 5074–5078.
[5] **Downing, S., Banack, S. a, Cox, P. a, et.al (2011).** *Toxicon : official journal of the International Society on Toxinology*, *58*(2), 187–94.
[6] **Faassen, E. J (2014).** *Toxins*, *6*(3), 1109–38.
[7] **Karlsson, O., Berg, A.-L., Andersson. M,. et.al (2012).** *Toxicological sciences : an official journal of the Society of Toxicology*, *130*(2), 391–404.
[8] **Pablo, J., Banack, S. a, Cox, P. a, et.al (2009).** *Acta neurologica Scandinavica*, *120*(4), 216–25.
[9] **Rao, S. D., Banack, S. A., Cox, P. A., et.al (2006).** *Experimental Neurology*, *201*(1), 244–252.
[10] **Wendee Holtcamp (2012).** *Environmental Health Perspectives,* 120(3), 110-116.